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CRITICAL CARE

Haemodilution-induced profibrinolytic state is mitigated by fresh-frozen plasma: implications for early haemostatic intervention in massive haemorrhage

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Background. Fibrinolysis contributes to coagulopathy after major trauma and surgery. We hypothesized that progressive haemodilution is responsible, at least in part, for increased fibrinolytic tendency of blood clot.

Methods. The study was performed in two parts. First, whole blood (WB) samples collected from six healthy, consented volunteers were diluted *in vitro* with either saline or fresh-frozen plasma (FFP) to 40% and 15% of baseline. We quantified factor levels related to coagulation and fibrinolysis, and measured endogenous thrombin generation in undiluted control plasma samples and in samples diluted with saline or FFP. Additionally, thromboelastometry was used to assess susceptibility to fibrinolysis after adding tissue plasminogen activator in undiluted WB samples and in samples diluted with saline before and after substitution of fibrinogen or FFP. Secondly, as a model of *in vivo* haemodilution, we evaluated the same parameters before and after operation in nine consented patients undergoing off-pump coronary artery bypass surgery.

Results. The dilution with saline caused dose-dependent decreases in plasma levels of coagulation and antifibrinolytic factors, and in thrombin generation. In FFP-supplemented samples, factor levels and thrombin generation were maintained within normal ranges. Fibrinolytic tendency was significantly higher after haemodilution with saline independent of fibrinogen substitution compared with FFP. Similarly, increased tendency for fibrinolysis was also observed in the *in vivo* haemodilution.

Conclusions. We demonstrated *in vitro* and *in vivo* that progressive haemodilution decreases endogenous antifibrinolytic proteins including α_2-antiplasmin and thrombin-activatable fibrinolysis inhibitor, resulting in increased fibrinolytic tendency. Therefore, early fluid replacement therapy with FFP might be advantageous after massive haemorrhage.

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Major trauma and surgery induce various degrees of vascular injury and exsanguination. In the case of massive blood loss requiring infusion of large amounts of i.v. fluids, coagulation factors are reduced to 30% of normal levels after loss of 1 blood volume, and to 15% after loss of 2 blood volumes.[1 2] The loss of multiple procoagulant factors is considered significant when prothrombin time (PT) is prolonged over 1.5 times of normal.[3] Although the diagnostic value of PT to predict bleeding is questionable,[4] fresh-frozen plasma (FFP) is frequently transfused in view of prolonged PT and haemorrhage as per the current guidelines.[5 6] However, there is a paucity of data on the regulation of fibrinolytic pathway and the role of FFP, if any, in major haemodilution. We have previously

demonstrated *in vitro* that clot resistance against tissue plasminogen activator (tPA)-induced fibrinolysis decreases after haemodilution.[7 8] Therefore, we speculated that fluid replacement using the crystalloid solution confers pro-fibrinolytic effects by diluting endogenous antifibrinolytic elements such as plasminogen activator inhibitor-1 (PAI-1), α_2-antiplasmin, thrombin-activatable fibrinolysis inhibitor (TAFI), and factor XIII (FXIII). Because FFP contains all the endogenous antifibrinolytic elements in plasma, it is plausible that clot stability against fibrinolysis would be better maintained if FFP were given early in massive traumatic haemorrhage.[9 10] Potential haemostatic mechanisms conferred by FFP have not been fully evaluated, whereas immunological and non-immunological complications of FFP transfusion have become obvious concerns.[6 11 12] We hypothesized that antifibrinolytic activity of FFP could be demonstrated by comparing whole blood (WB) haemodilution by normal saline *vs* FFP. In the first part of this study, levels of antifibrinolytic factors, thrombin generation, and thromboelastometric measurements of clot stability against tPA were compared after *in vitro* haemodilution (to about 40% and 15% of baseline values by normal saline and FFP). Similarly, in the second part of the study, increased fibrinolytic tendency of WB clot after moderate haemodilution was evaluated in patients undergoing off-pump coronary artery bypass (OPCAB) surgery in which antifibrinolytic therapy is not routinely implemented.

Methods

After institutional approval and informed written consent, blood samples were collected into 5 ml Vacutainer tubes (Beckton-Dickinson, Franklin Lakes, NJ, USA) containing sodium citrate 3.2% (0.5 ml) from six healthy volunteers (three females and three males, age between 24 and 63 yr) who had not received any drugs in the preceding 2 weeks and had no history of coagulopathy. In addition, pre- and postoperative blood samples from 10 patients undergoing OPCAB surgery involving more than three distal anastomoses were collected after informed written consent. All samples were obtained before heparin administration and after heparin reversal by protamine but before any other haemostatic intervention. OPCAB patients were chosen because dilutional effects are often present after surgery, but antifibrinolytics are not routinely used. Transfusion practice during OPCAB surgery was guided by the anaesthetist in charge and was not influenced by the study. One patient was referred to surgery with the use of cardiopulmonary bypass, and therefore was excluded, leaving nine patients for analyses (four females and five males, age between 50 and 79 yr).

During the experiments, all blood samples, diluted and undiluted, were either used immediately or centrifuged at $2000 \times g$ for 20 min (room temperature) to obtain platelet-poor plasma (PPP) which was stored at $-80^{\circ}C$ until analysis. For the *in vitro* model of haemodilution, WB was diluted 1:1 and 1:3 v/v with normal saline or allogeneic type-matched FFP, and haematocrit was restored to 23–25% by adding allogeneic type-matched RBC concentrate (haematocrit 52–63%). This dilution model has been described previously[7] and led to a total dilution of coagulation factors and platelets to about 2:5 (40%) and 1:6 (15–20%) of baseline, respectively. Fibrinogen has been shown to be protective against fibrinolysis.[7] Therefore, in WB samples diluted to 15% of baseline with saline, fibrinogen (Haemocomplettan® P; CSL Behring, Marburg, Germany) was substituted at a concentration of 2.7 g litre^{-1} which approximates the concentration of fibrinogen in FFP. These samples were used only for thromboelastometric measurements.

Laboratory measurements

We determined the following coagulation and antifibrinolytic markers and factors in PPP samples at baseline, and after dilution to 40% and 15% of baseline in the *in vitro* haemodilution model in healthy volunteers, and in pre- and postoperative samples from OPCAB patients: PT, international normalized ratio (INR), activated partial thromboplastin time (aPTT), fibrinogen, antithrombin, prothrombin (factor II), plasminogen, α_2-antiplasmin, and TAFI. Fibrinogen concentrations were determined using a modified Clauss method. Plasma prothrombin activity was determined using the one-stage clotting assay in factor II deficient plasma according to PT. Plasma AT, plasminogen, and α_2-antiplasmin activity were determined using a chromogenic method. These clotting and activity assays were performed on the STA Compact® analyzer (Diagnostica Stago, Parsippany, NJ, USA) using manufacturer's kits and direction. TAFI antigen was determined with an ELISA kit (Asserchrom® TAFI, Diagnostica Stago). Haemoglobin, haematocrit, and platelet count were measured by Coulter A^{c}T Series Analyzer (Coulter Corporation, Miami, FL, USA).

Thrombin generation

The calibrated automated thrombin generation system (Thrombinoscope™, Synapse BV, Maastricht, The Netherlands) was used to estimate the amount of thrombin generation based on the hydrolysis of a fluorogenic peptide by thrombin. Lag time, peak thrombin level, and endogenous thrombin potential were calculated as previously described by Hemker and colleagues.[13] For thrombin generation experiments, we used PPP samples at baseline and after dilution to 40% and 15% with either saline or FFP in the *in vitro* model and pre- and postoperative PPP samples from patients undergoing OPCAB surgery. Recalcified PPP samples were run in duplicate for thrombin generation triggered by 5 pM of relipidated tissue factor.

Thromboelastometric measurements

Thromboelastometry (ROTEM™; Pentapharm, Munich, Germany) measures the viscoelastic development from thrombin-mediated fibrin polymerization and platelet activation. Thromboelastometry is characterized by specific variables described earlier.[7] Using this technique, we collected the following parameters (Fig. 1): (i) coagulation time (CT; in seconds), which corresponds to the reaction time until clot formation; (ii) angle (α; in degrees), which reflects the rate of fibrin polymerization; (iii) maximal clot firmness (MCF; in millimetres), which refers to the maximal amplitude of the tracing and reflects the tensile strength of thrombus; (iv) lysis onset time (LOT; in seconds) defined as the time needed for clot firmness to decrease by 15% of MCF; and (v) lysis time (LT; in seconds) defined as the time needed for clot firmness to decrease by 90% of MCF. CT, angle, and MCF were obtained in the absence of tPA, whereas LOT and LT were evaluated in the presence of tPA.

The measurements in the blood samples from the same volunteer or patient were conducted simultaneously using three ROTEM™ analysers. All measurements were repeated twice at 37°C using 300 μl of diluted or undiluted WB after recalcification with 20 μl of 0.2 M $CaCl_2$ and activation with 10 μl of tissue factor (EXTEM®; Pentapharm) in the absence and presence of tPA (Alteplase®; Genentech, South San Francisco, CA, USA) at a concentration of 0.15 μg ml^{-1}.[7] In OPCAB patients, we additionally conducted the pre- and postoperative analyses in the presence of cytochalasin D (FIBTEM®; Pentapharm).

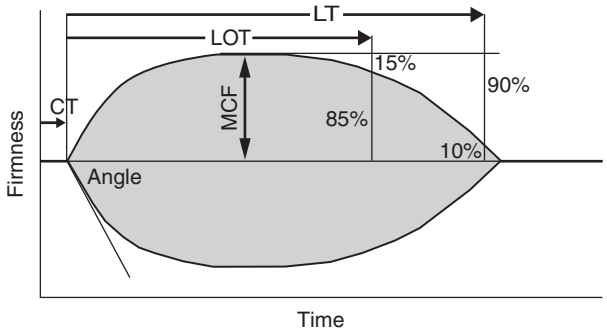

Fig 1 Thromboelastometric parameters: CT, angle, MCF, LOT, and LT are depicted. For more explanation see text.

Statistics

All experiments had $n \geq 6$ per condition, as this number of experiments is typically required to obtain a $\beta \geq 0.8$ and an $\alpha \leq 0.05$ for most thromboelastometric variables as demonstrated in previous *in vitro* investigations.[7] Data are expressed as mean (SD) or range where appropriate after testing by the Kolmogorov–Smirnov statistics for normal distribution. Data in the *in vitro* model of dilution were analysed using one-way analysis of variance (ANOVA) followed by Bonferroni's *post hoc* test. Data in the patient model of dilution were compared by paired *t*-test. A *P*-value of <0.05 was considered significant. All analyses were performed using SPSS® Version 16.0 (SPSS Inc., Chicago, IL, USA).

Results

Laboratory measurement

Table 1 shows blood cell counts and values for PT, INR, aPTT, fibrinogen concentration, and prothrombin, antithrombin, plasminogen, α_2-antiplasmin, and TAFI activity in the *in vitro* model of dilution. After dilution to 40% and 15% of baseline with saline, all clotting parameters decreased. Accordingly, PT and aPTT were prolonged to about 1.7- to 1.8-fold of baseline after dilution to 40% and to about 3- to 3.5-fold after dilution to 15%. In contrast, after dilution to 40% and 15% with FFP, all clotting parameters remained within normal range and were not

Table 1 *In vitro* dilution model in healthy volunteers. Data are mean (SD); per cent change relative to baseline value. Values for haemoglobin, haematocrit, and platelet count were identical for the dilution with saline and FFP. *P*-values were obtained by ANOVA followed by Bonferroni's *post hoc* test. **P*<0.05 *vs* baseline. FFP, fresh-frozen plasma; PT, prothrombin time; INR, international normalized ratio; aPTT, activated partial thromboplastin time; TAFI, tissue-activatable fibrinolysis inhibitor

	Baseline	Dilution to 40% of baseline		Dilution to 15% of baseline	
		With saline	With FFP	With saline	With FFP
Haemoglobin (g $litre^{-1}$)	157 (5)	91 (7); −42%*		89 (8); −43%*	
Haematocrit (%)	40.4 (1.9)	23.8 (1.9); −41%*		23.8 (2.1); −41%*	
Platelet ($\times 10^9$ $litre^{-1}$)	205 (31)	87 (17); −58%*		34 (5); −83%*	
PT (s)	11.8 (0.8)	21.5 (2.6); +82%*	12.4 (0.5); +5%	42.6 (6.2); +261%*	12.8 (0.4); +8%
INR	0.84 (0.07)	1.79 (0.26); +113%*	0.90 (0.04); +7%	3.94 (0.70); +369%*	0.93 (0.03); +11%
aPTT (s)	31.0 (4.5)	52.4 (11.5); +69%*	34.2 (2.7); +10%	90.0 (17.4); +190%*	36.7 (2.8); +18%
Fibrinogen (g $litre^{-1}$)	3.2 (0.5)	1.5 (0.2); −64%*	3.0 (0.2); −8%	0.5 (0.1); −84%*	2.8 (0.2); −12%
Prothrombin (%)	109 (10)	47 (5); −57%*	105 (8); −4%	19 (3); −83%*	98 (6); −10%
Antithrombin (%)	123 (10)	50 (7); −59%*	114 (2); −7%	28 (3); −77%*	100 (6); −19%*
Plasminogen (%)	98 (6)	42 (4); −57%*	90 (6); −8%	26 (1); −74%*	86 (4); −12%*
α_2-Antiplasmin (%)	95 (13)	38 (5); −58%*	89 (4); −6%	17 (2); −80%*	84 (4); −12%*
TAFI (μg ml^{-1})	9.7 (1.3)	3.7 (0.3); −62%*	9.5 (0.6); −2%	2.0 (0.1); −79%*	8.7 (1.0); −10%

different from baseline ($P>0.05$) except that antithrombin and plasminogen levels were slightly lower than at baseline. Similar trends were demonstrated in the *in vivo* model of haemodilution, presented in Table 2. Blood samples after OPCAB surgery were diluted to about 60% of baseline values by administration of saline (1800–4000 ml) and albumin 5% (500–1000 ml). Haemoglobin, platelet count, fibrinogen, and activities of prothrombin, antithrombin, and all antifibrinolytic factors were significantly decreased, whereas PT and aPTT values increased by about 1.3- to 1.4-fold relative to baseline values (all $P\leq0.010$).

Thrombin generation

In the *in vitro* model of dilution, lag time was 2.6 (0.5) min at baseline and did not change significantly after progressive dilution either with saline or with FFP (all $P=1.000$ *vs* baseline). In contrast, peak thrombin generation and endogenous thrombin potential (ETP) decreased significantly in saline diluted samples from 429 (29) and 2270 (123) nM at baseline to 249 (27) nM ($P<0.001$ *vs* baseline) and 2060 (78) nM ($P=0.034$ *vs* baseline) after dilution to 40% and to 136 (23) nM ($P<0.001$) and 1800 (151) nM ($P<0.001$) after dilution to 15% of baseline. After dilution with FFP to 40% and 15% of baseline, peak thrombin generation and ETP did not change significantly compared with baseline except that peak thrombin level was slightly reduced to 382 (22) nM after dilution to 15% ($P=0.025$ *vs* baseline). Figure 2 shows representative thrombin generation curves from the same volunteer in the *in vitro* model of dilution.

In plasma samples from OPCAB patients, lag time was 4.0 (1.6) min before surgery and 4.9 (2.8) min after surgery ($P=0.899$). Peak thrombin generation decreased from 427 (63) to 264 (91) nM ($P=0.001$), whereas ETP decreased non-significantly from 2580 (732) to 2100 (715) nM ($P=0.160$).

Table 2 *In vivo* dilution in patients undergoing off-pump coronary artery bypass (OPCAB) surgery. Data are mean (SD); per cent change relative to preoperative value. *P*-values were obtained by paired *t*-test. PT, prothrombin time; INR, international normalized ratio; aPTT, activated partial thromboplastin time; TAFI, tissue-activatable fibrinolysis inhibitor

	Preoperative	Postoperative	*P*-value
Haemoglobin (g litre^{-1})	134 (18)	91 (22); −32%	0.003
Haematocrit (%)	36.2 (4.5)	24.6 (6.6); −32%	0.002
Platelet ($\times10^9$ litre^{-1})	203 (81)	131 (66); −36%	<0.001
PT (s)	12.1 (1.2)	17.7 (5.0); +46%	0.010
INR	0.87 (0.11)	1.41 (0.5); +62%	0.013
aPTT (s)	34.7 (5.8)	43.6 (12.3); +26%	0.064
Fibrinogen (g litre^{-1})	4.5 (1.3)	2.6 (0.9); −43%	<0.001
Prothrombin (%)	104 (13)	60 (15); −42%	0.001
Antithrombin (%)	98 (8)	61 (10); −38%	<0.001
Plasminogen (%)	101 (18)	61 (15); −40%	<0.001
α_2-Antiplasmin (%)	90 (12)	51 (13); −43%	<0.001
TAFI (μg ml^{-1})	9.7 (2.0)	5.8 (1.6); −40%	<0.001

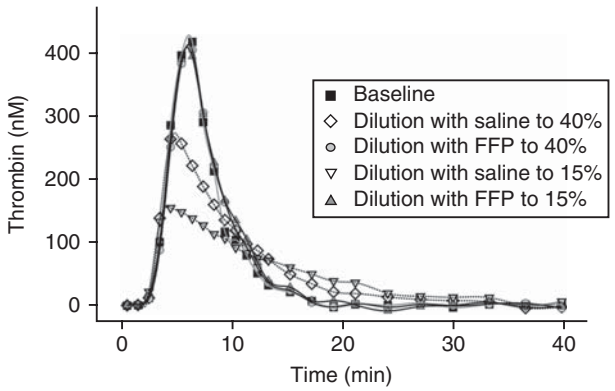

Fig 2 Representative thrombin generation curve in the PPP of a healthy volunteer after *in vitro* dilution: thrombin generation curves cannot be differentiated to baseline after dilution with FFP to 40% and 15%, whereas peak thrombin generation decreases dilution-dependently due to reduced concentration of procoagulant clotting factor. After dilution with saline, thrombin activity is sustained longer due to reduced antithrombin activity.

Thromboelastometry

For the *in vitro* model of dilution, CT, angle, and MCF values are shown in Table 3. CT was prolonged and angle decreased after dilution with saline, whereas these parameters were not significantly different from baseline after dilution with FFP. MCF was reduced with any dilution, but MCF values were significantly higher after dilution with FFP compared with dilution with saline ($P<0.001$). Addition of fibrinogen after dilution with saline improved CT, angle, and MCF (all $P<0.001$) and made these values similar to dilution to 15% with FFP (all $P\geq0.915$). Figure 3 shows LOT and LT as assessed by thromboelastometry. LOT and LT values after dilution with FFP were significantly longer compared with the corresponding dilution with saline (all $P<0.001$). Addition of fibrinogen did not significantly improve resistance against fibrinolysis ($P=1.000$ *vs* both dilution with saline).

Table 4 shows the findings in the OPCAB patients. In agreement with our *in vitro* dilution model, angle and MCF decreased by 8% and 13%, respectively, after surgery, whereas CT increased by about 1.4-fold. Further, LOT and LT decreased by about 45%. In the presence of cytochalasin D, we found a decrease in MCF of 33%, and decreases of LOT and LT of about 40%.

Discussion

We present the *in vitro* evidence that FFP administered with intention to replace acute blood loss confers better procoagulant and antifibrinolytic activity compared with treatment with saline or fibrinogen concentrate. Further, we demonstrate that haemodilution of endogenous antifibrinolytic factors is causally related to increased fibrinolytic tendency using both *in vitro* and *in vivo* models.

Table 3 Thromboelastometric parameters in the *in vitro* model of dilution. Values are mean (SD). *P*-values were obtained by ANOVA followed by Bonferroni's *post hoc* test. *$P<0.01$ *vs* baseline; §$P<0.01$ *vs* dilution to 15% with saline and fibrinogen. Thromboelastometric data were obtained after stimulation with EXTEM® in the absence of tPA. FFP, fresh frozen plasma; CT, coagulation time; MCF, maximal clotting firmness

	Baseline	Dilution to 40% of baseline		Dilution to 15% of baseline		
		With saline	With FFP	With saline	With FFP	With saline and fibrinogen
CT_{EXTEM} (s)	58 (7)	90 (15)*	56 (10)	150 (50)*,§	58 (9)	71 (11)
$Angle_{EXTEM}$ (°)	70 (5)§	57 (7)*	72 (5)§	33 (4)*,§	64 (7)	59 (10)*
MCF_{EXTEM} (mm)	57 (4)§	39 (4)*	52 (4)§	23 (2)*,§	40 (4)*	38 (2)*

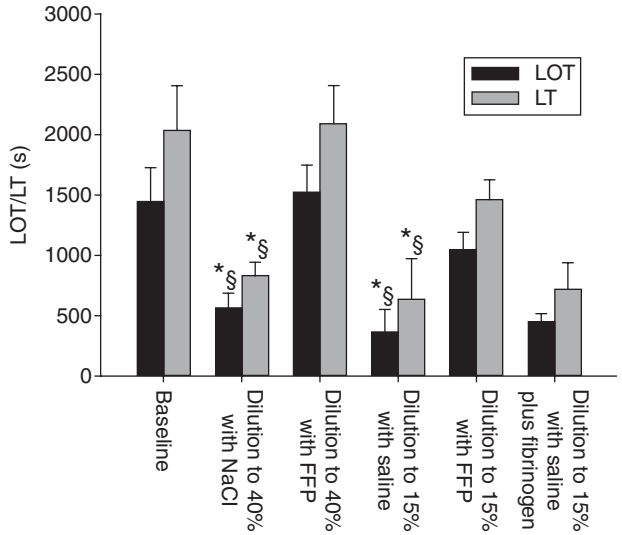

Fig 3 Thromboelastometric lysis parameters in the *in vitro* model of dilution: LOT and LT were significantly lower after dilution with saline compared with the corresponding dilution with FFP (*$P<0.001$). LOT and LT were comparable with baseline after dilution to 40% with FFP and decrease only after further dilution. Adding fibrinogen to the sample diluted with saline to 15% did not significantly increase LOT and LT (§$P=1.000$).

Table 4 Thromboelastometric parameters in patients undergoing off-pump coronary artery bypass (OPCAB) surgery. Data are mean (SD). *P*-values were obtained by paired *t*-test. Thromboelastometric data were obtained either in the absence (CT, angle, and MCF) or in the presence of tPA (LT and LOT) after stimulation with EXTEM® or FIBTEM® as specified. CT, coagulation time; MCF, maximal clotting firmness; LOT, lysis onset time; LT, lysis time

	Preoperative	Postoperative	*P*-value
CT_{EXTEM} (s)	53 (18)	82 (34)	0.004
$Angle_{EXTEM}$ (°)	76 (3)	70 (6)	0.001
MCF_{EXTEM} (mm)	67 (4)	58 (7)	<0.001
LOT_{EXTEM} (s)	1480 (488)	820 (426)	<0.001
LT_{EXTEM} (s)	2130 (773)	1140 (596)	<0.001
MCF_{FIBTEM} (mm)	21 (4)	14 (5)	<0.001
LOT_{FIBTEM} (s)	1030 (534)	664 (300)	0.005
LT_{FIBTEM} (s)	1540 (901)	901 (376)	<0.001

Coagulopathy after major trauma and surgery has multiple aetiologies including the loss (haemorrhage) and consumption of coagulation factors and platelets, haemodilution by fluid replacement, hypothermia, acidosis, and disseminated intravascular coagulation (DIC), that is, excess procoagulant and fibrinolytic activity.[14 15] Fibrinolysis is a normal physiological response necessary to dissolve excess fibrin that is formed within blood vessels, but re-bleeding may result from the premature breakdown of fibrin at the injury site.[14] Normally, several physiological mechanisms protect fibrin (clot) against fibrinolytic enzymes. Plasma concentration of α_2-antiplasmin is relatively high (70 $\mu g\ ml^{-1}$, 1 μM), and this plasmin inhibitor is rapidly cross-linked to fibrin α chains by thrombin-activated FXIIIa.[16] Congenital α_2-antiplasmin deficiency is thus known to cause severe bleeding tendency due to increased susceptibility to fibrinolysis.[17] Thrombin is also involved in the activation of TAFI zymogen (5 $\mu g\ ml^{-1}$, 75 nM). Activated TAFI cleaves carboxy-terminal lysine residues from the fibrin, and prevents the binding of plasminogen.[18] Although plasma concentration of PAI-1 is low (0.01 $\mu g\ ml^{-1}$, 200 pM), platelets release PAI-1 from their α-granules upon activation, rendering platelet-rich thrombi resistant to fibrinolysis.[19 20] Conversely, tPA can be released from the endothelium during acute stress in response to catecholamines, thrombin, vasopressin, and other humoral factors.[21 22] Thus, fibrinolytic pathway is relatively well preserved during haemodilution in conjunction with high baseline levels of plasminogen (200 $\mu g\ ml^{-1}$, 2 μM). Indeed, increased tPA-induced fibrinolysis was readily observed on thromboelastometry (Fig. 3) after WB was diluted to 40% of baseline with normal saline leading to mild thrombocytopenia (Table 1). In the similar dilution with FFP, clot stability against fibrinolysis was maintained until the level of dilution was increased to 15% with FFP leading to severe thrombocytopenia (platelet count $<40\times10^9$ $litre^{-1}$, Table 1). Endogenous thrombin generation did not change after dilution with FFP, but was substantially affected after dilution with saline. However, the decrease in peak thrombin was less than expected, that is, only about 60% of baseline in the dilution to 40% of baseline and about 33% after dilution to 15% of baseline. This is in contrast to grossly prolonged PT/aPTT values after only 40% dilution with saline (Table 1). PT/aPTT prolongations described here are in close agreement with Yuan and colleagues[3] who showed that PT/aPTT exceeded 1.5-times normal when coagulation factor levels were 50% of baseline. Unlike PT/aPTT, the thrombin generation assay is influenced not only by decreased procoagulant

factors, but also by lower antithrombin activity;[7] thus thrombin activity is sustained longer in plasma (Table 1). In this regard, reduced α_2-antiplasmin and TAFI levels but not thrombin generation (to activate TAFI) seem to contribute mainly to fibrinolytic tendency. In parallel with our *in vitro* findings, we observed that the WB clot was more prone to tPA-induced fibrinolysis after major fluid replacements during the multi-vessel OPCAB procedure (Tables 2 and 4).

Decreased fibrinogen level, thrombocytopenia, or both could explain our present *in vitro* and *in vivo* findings since low fibrinogen and low platelet count have been shown to increase tendency to fibrinolysis.[7 8] However, increasing the fibrinogen concentration did not significantly improve resistance against fibrinolysis in the *in vitro* model (Fig. 3). The interaction between fibrin(ogen) and activated platelets (via glycoprotein IIb/IIIa) seemed to confer some antifibrinolytic function because the extent of fibrinolysis was modestly increased in the presence of cytochalasin D, an inhibitor of platelet cytoskeletal reorganization (Table 4). Taken together, restoration of endogenous antifibrinolytic factors by FFP is presumably the main mechanism for clot protection in haemodilution-induced profibrinolytic state.

Clinical management of massive haemorrhage primarily consists of fluid resuscitation with crystalloids, colloids, or both in conjunction with red blood cell transfusion to ensure oxygen delivery.[2 5 6] Therefore, we used an *in vitro* model which includes haemodilution along with red blood cell supplementation to simulate clinically used resuscitation schemes.[7] The transfusion of haemostatic products used to be considered secondary to aggressive fluid resuscitation; however, this approach has been questioned recently.[23] Early intervention using FFP is increasingly utilized in massive transfusion protocols.[9 10] The empirical ratio of FFP:RBC transfusion of 1:1 seems efficacious in the retrospective analysis of massively transfused trauma cases in military and civilian studies.[10 24] Survival was significantly worse with low FFP:RBC transfusion ratio (e.g. $<$1:2) compared with high FFP:RBC ratio. However, it is difficult to delineate haemostatic mechanisms of FFP from clinical data which reported normalized PT/INR because this clotting time does not reflect the full extent of thrombin generation and fibrin polymerization.[25] Further, there are increasing clinical data supporting the use of fibrinogen concentrate to reduce blood loss after major surgery.[26–28] Therefore, the choice between FFP and fibrinogen in major haemorrhage remains controversial.

Our *in vitro* and *in vivo* data provide evidence that haemodilution by crystalloids renders clot susceptible to fibrinolysis due to dilution of endogenous antifibrinolytic factors. However, fibrinolysis may not be detectable unless thromboelastometry is performed using tPA to induce fibrinolysis (Tables 3 and 4). Fibrinogen *per se* can improve most thromboelastometric variables;[7] however, increased mass of fibrin is not protected against fibrinolysis unless endogenous antifibrinolytic elements are restored (Table 1). Levrat and colleagues[29] reported a high incidence of systemic fibrinolysis in trauma patients with higher injury severity scores. Although these investigators did not provide data on fluid resuscitation, lower platelet count and coagulation factor levels suggest that high-risk patients required more crystalloids for haemodynamic stabilization, and thus incurred more haemodilution. Further, haemodilution by colloids seems to increase susceptibility to fibrinolysis even more than crystalloids.[30] Our findings related to haemodilution also provide a biochemical basis for the clinical trial (Crash-2) which investigated the effectiveness of tranexamic acid in trauma patients.[31 32]

In cardiac surgery, antifibrinolytic agents are used routinely[33] because the extracorporeal circulation is presumed to induce hyperfibrinolysis via contact activation, and resultant coagulopathy. Antifibrinolytics have been shown to decrease blood product usage.[33] On the contrary, antifibrinolytic therapy is infrequently used in OPCAB surgery for fear of hypercoagulable state, and thrombotic complications. Our present data seem to support potential usefulness of antifibrinolytic agents in cases of extensive haemodilution. In agreement, several published double-blind randomized studies showed that tranexamic acid was effective in reducing postoperative blood loss and requirements for blood-derived products in OPCAB surgery.[34]

Our data also suggest that FFP supports endogenous antifibrinolytic activity if given in sufficient amounts as shown by reduced fibrinolysis on thromboelastometry. In the case of massive haemorrhage encountered in major trauma and surgery, anticoagulant (e.g. antithrombin) and antifibrinolytic factors rapidly decrease below 40% of baseline,[35] and systemic activation of thrombin and plasmin are likely to follow.[14 36] We speculate that early intervention with FFP before the occurrence of systemic fibrinolysis might mitigate haemorrhagic tendency described as early trauma-induced coagulopathy or profibrinolytic DIC.[10 36]

Obviously, there are safety concerns with the routine use of FFP which limit its therapeutic benefits.[11 12] First, FFP should not be considered as a primary fluid replacement therapy,[5 6] and high volume requirement of FFP to raise factor levels can cause volume overload. Secondly, there is a potential, although low, risk of viral transmission with FFP. Such a risk may be further reduced in the future as more virus-inactivated plasma products become available.[37] The incidence of transfusion-related acute lung injury has recently decreased after the adaptation of male only donor policy for FFP.[38] However, increased risks for multi-organ failure and sepsis associated with FFP have been reported based on the retrospective analysis of transfusion data.[12] It is prudent to prospectively evaluate the potential efficacy and safety of FFP in preventing uncontrolled thrombin generation and hyperfibrinolysis in early trauma-induced coagulopathy.

In conclusion, we demonstrated a haemodilution-induced profibrinolytic state *in vitro* and *in vivo*. Although the extent of fibrinolysis occurring in individual cases is difficult to determine, blood clot is likely more susceptible to localized fibrinolysis, which can worsen bleeding related to thrombocytopenia, low fibrinogen, and other factor deficiencies after major haemorrhage. Early fluid replacement therapy using FFP might maintain appropriate levels of clotting factors, and also improve endogenous antifibrinolytic activity in the absence of exogenous antifibrinolytic therapy. Our *in vitro* finding on haemodilution-related fibrinolysis was strongly supported by the *in vivo* haemodilution data during OPCAB surgery. Further clinical studies are warranted to confirm our findings and to prospectively investigate the safety and efficacy of early replacement therapy using FFP *vs* a combination of fibrinogen concentrate and tranexamic acid.

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